Mar [illegible]

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FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
14:1[illegible] MAY 2[illegible]
L[illegible] 14 DUP REM L12 (0 DUPLICATES REMOVED)
L14 14 S L13 NOT L11

=> s [illegible]
L1 [illegible] SEA TSIEN R? AU
L2 1 SEA CAMPBELL R?/AU
L3 [illegible] SEA L1 OR L2
L4 14 SEA TANDEM(5A) FLUORESCENT (5A) PROTEIN#
L5 1 SEA L4 AND L3
L6 111 SEA DIMER?(5A) FLUORESCENT (5A) PROTEIN#
L7 10 SEA L6 AND L3
L11 [illegible] SEA FUSION OR FUSED OR CHIMERA? OR CHIMERIC? (5A) FLUORESCENT (5A)
PROTEIN#
L13 11 SEA L11 AND L3
L12 14 SEA L5 OR L7 OR L11, AND (GREEN OR GFP)
L22 14 DUP REM L12 (18 DUPLICATES REMOVED)

=> d ibib abs 122 1-14

L22 ANSWER 1 OF 14 HCAPLUS COPYRIGHT 2[illegible] ACS
ACCESSION NUMBER: [illegible]:[illegible] HCAPLUS
DOCUMENT NUMBER: 138:207491
TITLE: Monomeric and **dimeric** DsRed **fluorescent protein** variants and DNAs and methods for preparing and using the proteins
INVENTOR(S): **Tsien, Roger Y.; Campbell, Robert E.**
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 67 pp., Cont.-in-part of U.S. Pat. Appl. 2003 32,488.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003[illegible]9535	A1	2003032[illegible]	[illegible] 2002-121255	2002041[illegible]
US 2[illegible]3 [illegible]2068	A1	2[illegible]30213	[illegible] 2001-[illegible]66538	2[illegible]10524

PRIORITY APPLN. INFO.: [illegible] US [illegible]-[illegible]4[illegible] A2 2[illegible]1[illegible]2[illegible]
US 2001-866538 A2 20010524

AB The present invention relates to monomeric and **dimeric** forms of Anthozoan **fluorescent proteins**, esp. Discosoma DsRed. The invention also relates to methods of making such **fluorescent protein** monomers and **dimers** using, for example, error-prone PCR. The improved fluorescent proteins may be used for assaying transcription as well as for anal. of in vivo localization or trafficking of proteins. Thus, DsRed variants which no longer tetramerize but instead form dimers, or exist as monomers were produced. The monomeric variant mRFP1 matures rapidly and has minimal emission when excited at wavelengths optimal for **GFP**. Application of mol. evolution paired with the DsRed system to mutagenesis of **GFP** should result in **GFP** mutants which no longer dimerize.

Search completed by David Schreiber [illegible]-4292

L[illegible] ANSWER [illegible] OF 14 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2[illegible]:[illegible] HCAPLUS
DOCUMENT NUMBER: [illegible]
TITLE: **Dimeric fusion** [illegible] **fluorescent proteins** showing low [illegible]
INVENTOR(S): **Tsien, Roger Y.**; **Campbell, Robert E.**
PATENT ASSIGNEE(S): UCSD
SOURCE: U.S. Pat. Appl. Publ., 42 pp., Cont.-in-part of U.S. Ser. No. [illegible].
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: [illegible]
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
[illegible]	A1	[illegible]	[illegible]	[illegible]
[illegible]	A2	[illegible]	[illegible]	[illegible]
[illegible]	A1	[illegible]		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
US 20030[illegible] A1 20030327 US 2002-121258 20020410
PRIORITY APPLN. INFO.: US 2001-794306 A2 20010226
US 2001-866538 A 20010524

AB Fluorescent proteins, such as **green** fluorescent protein, that show lower degrees of oligomerization are described for anal. use. The non-oligomerizing derivs. are **fusion proteins** of **fluorescent proteins** including a naturally occurring **green** [illegible]-fluorescent **protein**, and **fluorescent protein** or derivs. that are **dimers** of the **fluorescent protein** connected by a linker peptide. The protein may also carry other modifications, such as in surface residues, that prevent oligomerization without a significant impact on fluorescence properties. Also provided is a fusion protein, which includes a non-oligomerizing fluorescent protein linked to at least one polypeptide of interest. Chimeric genes encoding these fusion proteins and expression constructs and hosts for the genes are also described.

L[illegible] ANSWER [illegible] OF 14 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:[illegible] HCAPLUS
DOCUMENT NUMBER: 137:[illegible]
TITLE: Non-oligomerizing fluorescent proteins and their uses
INVENTOR(S): **Tsien, Roger Y.**; Baird, Geoffrey S.; **Campbell, Robert E.**; Zacharias, David A.
PATENT ASSIGNEE(S): The Regents of the University of California, USA
SOURCE: PCT Int. Appl., [illegible] pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English

Page [illegible]

FAMILY AVAIL. WITH 1 UNIT:
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

US: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

[illegible] A1 [illegible] [illegible] [illegible]

PRIORITY APPLN. INFO.: [illegible] A [illegible]
[illegible] A [illegible]
[illegible] A [illegible]

AB Non-oligomerizing fluorescent proteins, which are primarily monomeric, and oligomers of a fluorescent protein, in which are derived from a fluorescent protein having at least one mutation that promotes or minimizes the ability of the fluorescent protein to oligomerize, are provided. The non-oligomerizing fluorescent protein can be derived from a naturally occurring **green** fluorescent protein, a red fluorescent protein, or other fluorescent protein, or a fluorescent protein derivative thereto. Also provided is a fusion protein, which includes a non-oligomerizing fluorescent protein linked to at least one polypeptide of interest. In addn., a polynucleotide encoding a non-oligomerizing fluorescent protein is provided, as is a recombinant nucleic acid mol., which includes polynucleotide encoding a non-oligomerizing fluorescent protein operatively linked to at least a second polynucleotide. Vectors and host cells contg. such polynucleotides also are provided, as are kits contg. one or more non-oligomerizing fluorescent proteins or encoding polynucleotides or constructs derived therefrom. Further provided are methods of making and using the proteins and polynucleotides, e.g., to det. pH in cells or biol. tissues, to det. the presence or activity of enzymes, to identify agents or conditions which regulate the activity of an expression control sequence, and to identify interactions of a first and second mol. Thus, residues 206-alanine, 221-leucine, and 223-phenylalanine were shown to be involved in oligomerization of **GFP**. Substitution of charged amino acids (e.g., lysine, arginine) for these residues prevented oligomerization.

L44 ANSWER 4 OF 19 MEDLINE DUPLICATE 1
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: A monomeric red fluorescent protein.
AUTHOR: **Campbell Robert E**; Tour [illegible]; Palmer Amy E; Steinbach Paul A; Baird Geoffrey S; Zacharias David A; **Tsien Roger Y**
CORPORATE SOURCE: Department of Pharmacology, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA.
CONTRACT NUMBER: [illegible]
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, [illegible]

[illegible] computer by Patent Threshold [illegible]

[illegible]

[illegible] Journal Name: [illegible]. ISSN: [illegible].
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: [illegible] Priority Journals
OTHER SOURCE: GENBANK-AF[illegible]; GENBANK-AF[illegible]; GENBANK-AF[illegible]
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated in STN: [illegible]
Entered Medline: [illegible]

AB [illegible] fluorescent proteins (FPs) have display[illegible] in [illegible] quaternary structure, including the weak tendency [illegible] Aequorea **green fluorescent protein** (**GFP**) to **dimerize**, the [illegible] dimerization of Renilla **GFP**, and the [illegible] dimerization of the red fluorescent protein from Discosoma (DsRed). Both the weak dimerization of Aequorea **GFP** has [illegible] improved its performance as an indispensable [illegible] cell biology, the [illegible] dimerization of DsRed has greatly hindered its use as a potentially [illegible] fusion tag. We present here the stepwise [illegible] DsRed to a dimer and then either to a genetic fusion of two copies of the protein, i.e., a tandem dimer, or to a true monomer designated mRFP1 [illegible] red fluorescent protein. Each subunit interface was disrupted by hundreds [illegible] mutations, which initially crippled the resulting protein, but [illegible] could be reversed by random and directed mutagenesis [illegible] mutations in the dimer and to [illegible] mRFP1. Part [illegible] the [illegible] protein combining [illegible] mRFP1 forms fully functional [illegible] fusions, whereas analogous [illegible] the tandem and dimer failed. Although mRFP1 [illegible] maturation [illegible], [illegible] yield, [illegible] photostability than DsRed, mRFP1 matures [illegible] times faster, and [illegible] brightness in living cells. In addition, the excitation and emission peaks [illegible] mRFP1, 584 and 607 nm, are approximately 25 nm red-shifted from DsRed, which should confer greater tissue penetration and spectral separation from autofluorescence and other fluorescent proteins.

L21 ANSWER 2 OF 14 MEDLINE DUPLICATE 2
ACCESSION NUMBER: [illegible] 12049561 MEDLINE
DOCUMENT NUMBER: [illegible]4700 PubMed ID: 11988576
TITLE: Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells.
COMMENT: Comment in: Science. 2002 May 3;296(5569):855-7
AUTHOR: Zacharias David A; Violin Jonathan D; Newton Alexandra C; **Tsien Roger Y**
CORPORATE SOURCE: Department of Pharmacology, Biomedical Sciences Graduate Program, and, Howard Hughes Medical Institute, University of California, San Diego, La Jolla, CA 92093-0647, USA.
CONTRACT NUMBER: [illegible] GM07752 (NIGMS)
[illegible]4441 (NINDS)
[illegible] (NINDS)
SOURCE: SCIENCE, (2002 May 3) 296 (5569) 913-6.
Journal code: [illegible]. ISSN: [illegible].
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: [illegible] Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated in STN: [illegible]
Entered Medline: [illegible]
AB Many [illegible] associated with the plasma membrane are known to [illegible] partition

[illegible] search completed by David Schreiber [illegible]

Page ...

... fluorescent proteins to dimerize

L22 ANSWER ... OF ... BIOSIS COPYRIGHT ... BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: ...
DOCUMENT NUMBER: ...
TITLE: A ... bipartite motif is required for the localization of inhibitory kappaB-like IkappaBL protein to nuclear speckles.
AUTHOR(S): Kempler, Jennifer L.; Brown, Stephanie B.; Jarvis, Christopher M.; **Campbell, R. Duncan (1)**
CORPORATE SOURCE: (1) Functional Genomics Group, MRC UK HGMP Resource Centre, Hinxton, Cambridge, CB1 1SB: rdc@hgmp.mrc.ac.uk UK
SOURCE: BMC Genomics, 21 February, ... 2, No. 1, pp. 4pp.-4pp. http://www.biomedcentral.com/. print. ISSN: ...-...
DOCUMENT TYPE: Article
LANGUAGE: English
AB The inhibitory kappaB (IkappaB)-like (IkappaBL) gene is located within the class III region of the MHC in human chromosome 6. Primary analysis of the predicted amino acid sequence of the human IkappaBL protein revealed three putative functional domains; a 5-ankyrin repeat sequence, which are similar to the second and third ankyrin repeats of the nuclear factor kappaB (NF-kappaB) protein; three PEST sequence motifs (a sequence that is rich in proline, serine, aspartic acid and threonine residues), which are also found in other IkappaB family members; and a C-terminal leucine zipper-like motif. In the present study we have identified a novel bipartite motif, which is required for nuclear localization of the IkappaBL protein. Analysis of IkappaBL-specific transcripts revealed the existence of a widely expressed splice variant form of IkappaBL (IkappaBLsv1), which lacks the amino acid sequence SELEDEWDYEMRFH (where single-letter amino-acid notation has been used). Interestingly, translation of IkappaBL mRNA in vitro was found to initiate predominantly from the second available methionine, thereby resulting in the disruption of the predicted N-terminal PEST sequence. Also, transient expression of T7-epitope-tagged IkappaBL and IkappaBLsv1 proteins in mammalian cells showed that both proteins were targeted to the nucleus, where they accumulate in nuclear speckles. To define the protein domains required for nuclear import and subnuclear localization, a complementary set of deletion mutants and enhanced **green** fluorescent protein-IkappaBL domain fusions were expressed in mammalian cells. Data from these experiments show that a combination of the ankyrin-repeat region and an adjacent arginine-rich sequence are necessary and sufficient for both nuclear import and speckle localization.

L22 ANSWER ... OF ... HCAPLUS COPYRIGHT ... ACS
ACCESSION NUMBER: ...:...
DOCUMENT NUMBER: ...:...
TITLE: Modified **green** fluorescent protein ...
Appl ...
INVENTOR(S): **Tsien, Roger Y.**; Heim, Roger

Search completed by Carol Schreiber ...-...

PATENT ASSIGNEE(S): Regents of the University of California, USA
SOURCE: U.S., 22 pp., Cont.-in-part of U.S. 5,625,048.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US [illegible]	B1	[illegible]	US 1997-[illegible]	1997[illegible]
US 5,777,079	A	19980707	US 1994-337915	19941110
CA 2343[illegible]	AA	19960808	CA 1995-2205006	19951113
WO 9623810	A1	19960808	WO 1995-US14692	19951113
W: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
EP 1[illegible]	A2	20010606	EP 2001-105011	19951113
EP 1[illegible]	A3	20020918		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE				
US [illegible]	A	19980707	US 1996-753143	19961120

PRIORITY APPLN. INFO.: US 1994-337915 A2 19941110
WO 1995-US14692 W 19951113
CA 1995-2205006 A3 19951113
EP 1995-939896 A3 19951113
US 1996-753143 A3 19961016

AB Modifications in the sequence of Aequorea wild-type **GFP** provide products having markedly different excitation and emission spectra from corresponding products from wild-type **GFP**. In one class of modifications, the product derived from the modified **GFP** exhibits an alteration in the ratio of two main excitation peaks obsd. with the product derived from wild-type **GFP**. In another class, the product derived from the modified **GFP** fluoresces at a shorter wavelength than the corresponding product from wild-type **GFP**. In yet another class of modifications, the product derived from the modified **GFP** exhibits only a single excitation peak and enhanced emission relative to the product derived from wild-type **GFP**.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 6 OF 19 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2001429679 MEDLINE
DOCUMENT NUMBER: 21369963 PubMed ID: 11387331
TITLE: Reducing the environmental sensitivity of yellow fluorescent protein. Mechanism and applications.
AUTHOR: Griesbeck O; Baird G S; **Campbell R E**; Zacharias D A; **Tsien R Y**
CORPORATE SOURCE: Howard Hughes Medical Institute, University of California, San Diego, La Jolla, California 92093-0647, USA.
CONTRACT NUMBER: [illegible]CA23100-16 (NCI)
[illegible] (NINDS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, [illegible] Jul 27 276 (31) 29188-94.
Journal code: [illegible]. ISSN: [illegible].

Search completed by David Schreiber [illegible]

Mar [illegible], [illegible]

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; JOURNAL ARTICLE
LANGUAGE: English
FILE SEGMENT: Biology Digest
FILE LOAD DATE: 960-1997
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated in STN: [illegible]
Entered Medline: [illegible]

AB [illegible] **green** [illegible] protein [illegible] YFP [illegible] spectral [illegible] mutations [illegible] potentially [illegible] and [illegible] [illegible] [illegible] translocation [illegible] [illegible] to [illegible] protein-protein interactions. However, previous YFPs show excessive pH sensitivity, which is intermediate, pH photostability, or poor expression at 37° degrees. A protein evolution experiment in mammalian cells has produced a new YFP named Venus, in which the mutation F46L partners a much more pH [illegible] than previous YFPs, indifference to chloride, twice the photostability of previous YFPs, and much better expression at 37 degrees C and in fusions. The halide resistance is explained by a [illegible]-Å X-ray crystal structure of Venus, revealing that the methionine side chain fills what was once a large halide-binding cavity adjacent to the chromophore. Insertion of Venus within Venus or **fusion** of Venus to **fluorescent protein**, calmodulin, a calmodulin-binding peptide and Venus has generated improved calcium indicators. These indicators can be targeted to multiple subcellular locations and have permitted the first single-cell imaging of free [illegible] in the Golgi. Venus is superior to all previous YFPs except Citrine for pH or halide sensitivity in mammals and is particularly advantageous within genetically encoded fluorescent indicators of physiological signals.

L22 ANSWER [illegible] OF 17 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:657326 HCAPLUS
DOCUMENT NUMBER: 136:321466
TITLE: Fluorescence resonance energy transfer analysis of cell surface receptor interactions and signaling using spectral variants of the **green** fluorescent protein
AUTHOR(S): Chan, Francis Ka-Ming; Siegel, Richard M.; Zacharias, David; Swofford, R.R.; Holmes, Kevin L.; **Tsien, Roger Y.**; Lenardo, Michael J.
CORPORATE SOURCE: Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, 20892-1892, USA
SOURCE: Cytometry (2001), 44(4), 361-368
CODEN: CYTODQ; ISSN: 0196-4763
PUBLISHER: Wiley-Liss, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Fluorescence resonance energy transfer (FRET) is a powerful technique for measuring mol. interactions at Angstrom distances. We present a new method for FRET that utilizes the unique spectral properties of variants of the **green** fluorescent protein (**GFP**) for large-scale anal. by flow cytometry. The proteins of interest are used in transfection. The cyan fluorescent protein (CFP) and the yellow fluorescent protein (YFP). FRET between these differentially tagged fusion proteins is analyzed using a dual-laser FACSVantage cytometer. We show that homotypic interactions between individual receptor chains of tumor necrosis factor receptor (TNFR) family members can be detected as FRET from YFP-tagged receptor chains to YFP-tagged receptor chains. Nonequivalent mol.

Search completed by David Schreiber [illegible]-4292

Mar [illegible]

[illegible] by interaction is FRET between cyan and yellow [illegible] within the intracellular or extracellular regions of the protein. The [illegible] of the assay to be detected by the change in FRET between interacting fluorophores that are fused to. [illegible] GFP is the most commonly used FRET pair. Fluorescence lifetime [illegible] by the most commonly FRET pair. Fluorescence lifetime is [illegible] FRET allows [illegible] within the intracellular environment as well as [illegible] within the cell. Thirdly, by using FRET allows the [illegible] cell. [illegible] Alternatively, flow cytometry allows [illegible] be carried out on a cell-by-cell basis [illegible].

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 1 OF 1 WPIX COPYRIGHT 2004 DERWENT INFO
ACCESSION NUMBER: 1999-[illegible] WPIX
DOCUMENT NUMBER: 114:1442[illegible]
TITLE: **Green** fluorescent protein mutants with stability, ligand-binding, and peptide insertion properties and methods
INVENTOR(S): **Tsien, Roger Y.**; Baird, Geoffrey A.
PATENT ASSIGNEE(S): Regents of the University of California, The, USA
SOURCE: PCT Int. Appl., 94 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 6
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2[illegible]7[illegible]5	A2	2[illegible]1	WO 2[illegible]-US13[illegible]4	2[illegible]17
WO 2[illegible]1[illegible]5	A3	2[illegible]2[illegible]4		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: BW, GH, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

[illegible] A1 [illegible] [illegible] 1[illegible]-[illegible]4 1[illegible]1[illegible]
AU [illegible] A [illegible] [illegible] [illegible]-[illegible] [illegible]
WO [illegible] A1 2[illegible]1[illegible] WO [illegible]-[illegible]4 [illegible]

PRIORITY APPLN. INFO.:
US 1[illegible]-31[illegible]19 A 1[illegible]
US 1[illegible]-31[illegible]20 A 1[illegible]
WO 2[illegible]-US13654 W 2[illegible]

AB The present invention provides polypeptide and polynucleotides encoding fluorescent indicators having inserted within a fluorescent moiety a second polypeptide. The proteins are derivs. that are not normally fluorescent or exhibit FRET coupling. -Binding of a ligand to the second polypeptide in a mammalian cell and an increase in the fluorescence intensity of the protein. Also provided are methods of using the fluorescent indicators. The invention permits the detection and

[illegible] from computer by David Schreiber [illegible]-4[illegible]2

Page [illegible]

[illegible] duplicated.

L[illegible] ANSWER [illegible] OF [illegible] HCAPLUS [illegible] COPYRIGHT [illegible] ACS
ACCESSION NUMBER: [illegible]:[illegible] HCAPLUS
DOCUMENT NUMBER: [illegible]:[illegible]
TITLE: Fluorescent protein sensors for measuring the pH of a biological sample
INVENTOR(S): **Tsien, Roger Y.**; [illegible], Xian; Miyawaki, Atsushi; Remington, S. James
PATENT ASSIGNEE(S): The Regents of the University of California, USA; The [illegible] Institute Of Molecular Medicine; [illegible] Higher Education
SOURCE: [illegible], [illegible] pp., Cont.-in-part of U. S. Ser. No. [illegible].
CODEN: [illegible]
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: [illegible]
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
[illegible]	A	[illegible]	[illegible]	[illegible]
[illegible]	A	[illegible]	[illegible]	[illegible]
[illegible]	A2	[illegible]	[illegible]	[illegible]
[illegible]	A3	[illegible]		

PRIORITY APPLN. INFO.: [illegible]

AB Disclosed are fluorescent protein sensors for measuring the pH of a sample, nucleic acids encoding them, and methods of use. The preferred fluorescent protein sensors are variants of the **green** fluorescent protein (**GFP**) from Aequorea victoria. Also disclosed are compns. and methods for measuring the pH of a specific region of a cell, such as the mitochondrial matrix or the Golgi lumen. Enhanced **green** and enhanced yellow fluorescent proteins (recombinantly prepd.) were used as indicators of pH in the cytosol or nucleus. To construct proteins to use as pH indicators in the [illegible] apparatus, polynucleotides encoding the [illegible]-N-terminal amino acids of the type II membrane-anchored protein galactosyltransferase were ligated to polynucleotides encoding the mutant **green** fluorescent proteins.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L[illegible] ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:140011 HCAPLUS
DOCUMENT NUMBER: 132:81423
TITLE: A genetically encoded, fluorescent indicator for cyclic AMP in living cells
AUTHOR(S): Zaccolo, Manuela; De Giorgi, Francesca; Cho, Charles Y.; Feng, Limin; Knapp, Tim; Negulescu, Paul A.; Taylor, Susan S.; **Tsien, Roger Y.**; Pozzan, Tullio
CORPORATE SOURCE: Department of Experimental Biomedical Sciences, University of Padova, Padua, 35121, Italy
SOURCE: Nature Cell Biology (2000), 2(1), 25-29
CODEN: NCBIFN; ISSN: 1465-7392
PUBLISHER: Macmillan Magazines Ltd

Search completed by David Johnsonbaugh [illegible]-4234

DOCUMENT TYPE: Journal
LANGUAGE: English
AB The VAMP family of several highly conserved proteins within cells, and studies in the area. In this report, we present data on a potential role in many cellular processes. Here we describe a new method for the identification of VAMP in living cells. By fusing the VAMP effector protein A with the cytoplasmic **green** fluorescent protein, we have generated a probe in which the fluorescence is efficiently quenched when the fusion protein is exposed to the lumen of synaptic vesicles. This quenching may be reversible. The VAMP
VAMP in live .
REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

[illegible]
ACCESSION NUMBER: [illegible]
DOCUMENT NUMBER: [illegible]
TITLE: Monitoring exocytosis by quenching of **green** fluorescent protein fluorescence in the lumen of synaptic vesicles.
AUTHOR(S): [illegible]; Tsien, R. W.
CORPORATE SOURCE: [illegible]
SOURCE: [illegible]
[illegible]
[illegible]
[illegible]
DOCUMENT TYPE: [illegible]
LANGUAGE: English
SUMMARY LANGUAGE: English
AB FM dyes allow fluorescence imaging of active presynaptic terminals and provide valuable insights into dynamics of vesicular turnover, but are not ideally suited for studying the kinetics of exocytosis. FM1-43, for example, requires uptake to departition from the membrane. To selectively monitor exocytosis independent of endocytosis we have developed a new approach based on the quenching of a fluorophore permanently held within the vesicle lumen by a probe that enters vesicles upon **fusion**. The fluorophore is **green** **fluorescent protein** (GFP) associated with the lumenal domain of VAMP (synaptobrevin-2), introduced by transfection, and the quencher is bromophenol blue (BPB), a small, hydrophilic molecule. In quenching experiments, boutons were first exposed to BPB in Ca2+-free Tyrode's to eliminate fluorescence of any VAMP-**GFP** that had somehow strayed on the plasma membrane. Subsequent exposure to 90 mM K+/2 mM Ca2+ to induce voltage-gated Ca2+ entry caused sharp drop in **GFP** fluorescence (n=7), with biphasic kinetics reminiscent of [illegible] of FM1-43. In contrast, high K+/Ca2+ stimulation in the presence of BPB failed to decrease the fluorescence of puncta, even though a later repeat of the same challenge in BPB caused clear decreases in fluorescence in the same puncta (n=4). Evidently, pH increases during exocytosis had no significant effect on the fluorescence of this **GFP** construct. When BPB-loaded boutons were subjected to high K+/Ca2+ stimulation in the absence of BPB, the fluorescence of puncta suddenly increased, as expected if BPB had escaped from vesicles undergoing fusion. One advantage of this approach is that VAMP-**GFP** can mark boutons even if they are functionally silent. The exocytosis of quencher-loaded vesicles causes a fall in signal to increase, [illegible] of signals again from [illegible]. Extending this approach to quenchers of different size

[illegible] completed by David Zumbiber [illegible]

[illegible]

[illegible]

L[illegible] ANSWER [illegible] OF [illegible] HCAPLUS [illegible] COPYRIGHT [illegible] ACS
ACCESSION NUMBER: [illegible]:[illegible] HCAPLUS
DOCUMENT NUMBER: [illegible]:[illegible]
TITLE: **Green** fl[illegible]escent proteins for m[illegible]ring intr[illegible]ular pH in a[illegible]logical samples
INVENTOR(S): **Tsien, Roger Y.**; Li [illegible], Xiao; Wa[illegible], Bermuda M.
PATENT ASSIGNEE(S): The Regents of the University [illegible], USA; [illegible]
SOURCE: PCT Int. Appl., [illegible] pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO [illegible]	A2	[illegible]	WO [illegible]-US[illegible]	[illegible]
WO [illegible]	A3	[illegible]		

DESIGNATED STATES: W: AU, B[illegible], BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VC, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
WO [illegible] A [illegible] WO [illegible]-US[illegible] [illegible]
WO [illegible] A [illegible] WO [illegible]-US[illegible] [illegible]
IBI PRIORITY APPLN. INFO.: US [illegible]-[illegible] A [illegible]
US [illegible]-[illegible] A [illegible]

AB [illegible] **green** fluorescent protein sensors, including their protein and encoding nucleotide sequences, which show reversible fluorescence changes of physiol. pH ranges for measuring the pH of a sample, and methods of use for measuring the pH of specific intracellular regions. The preferred fluorescent protein sensors are variants of the **green** fluorescent protein (**GFP**) from Aequorea victoria. Also disclosed are compns. and methods for measuring the pH of a specific region of a cell, such as the mitochondrial matrix or the Golgi lumen. Some sequences listings were not provided by the authors.

L[illegible] ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT [illegible] ACS
ACCESSION NUMBER: [illegible]:[illegible] HCAPLUS
DOCUMENT NUMBER: [illegible]:[illegible]
TITLE: **Tandem fluorescent protein** constructs and their preparation [illegible] enzyme assays
INVENTOR(S): **Tsien, Roger Y.**; Heim, Roger; Cubitt, Andrew
PATENT ASSIGNEE(S): The Regents of the University of California, USA; Aurora Biosciences Corporation
SOURCE: U.S., [illegible] pp., Cont.-in-part of U.S. Ser. No. [illegible], [illegible].
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US [illegible]	A	[illegible]	US [illegible]-[illegible]	[illegible]
WO [illegible]	A1	[illegible]	WO [illegible]-US[illegible]	[illegible]

Search completed by David J Kreiber [illegible]

L[illegible]

[illegible]

AB [illegible] **tandem fluorescent protein** [illegible] a third fluorescent protein moiety, an insert in the second protein moiety and a linker moiety that couples the first and inserted moieties. The third and inserted moieties exhibit fluorescence energy transfer which is eliminated upon cleavage. The [illegible] is useful in assays. Mutant **green** [illegible] GFP. Polyhistidine tagged **tandem green** [illegible] **fluorescent proteins** were [illegible] with cleavage sites for many proteases. Cleavage expts. were done with trypsin, enterokinase and factor Xa.

REFERENCE COUNT: [illegible] THERE ARE [illegible] CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L[illegible] ANSWER [illegible] OF [illegible] MEDLINE [illegible] DUPLICATE 4
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: [illegible] circular permutation and receptor insertion within **green** [illegible] **fluorescent proteins**.
AUTHOR: [illegible] Baird G S; Zacharias D A; **Tsien R Y**
CORPORATE SOURCE: [illegible] Department of Pharmacology, University of California at San [illegible], La Jolla, CA [illegible], USA.
CONTRACT NUMBER: [illegible] (NINDS)
SOURCE: [illegible] PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, [illegible] Sep [illegible] [illegible] 11441-6.
Journal code: [illegible]. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB Many areas of biology and biotechnology have been revolutionized by the ability to label **proteins** genetically by **fusion** to the Aequorea **green fluorescent protein** **GFP**. In previous fusions, the **GFP** has been treated as an indivisible entity, usually appended to the amino or carboxyl terminus of the host protein, occasionally inserted within the host sequence. The tightly interwoven, three-dimensional structure and intricate posttranslational self-modification required for chromophore formation would prevent the major rearrangements or insertions within **GFP** would prevent fluorescence. However, we now show that several rearrangements of GFPs, in which the amino and carboxyl portions are interchanged and rejoined with a short spacer connecting the original terminal, still have the fluorescence. These circular permutations have altered pKa values and orientations of the chromophore with respect to the protein surface. Furthermore, certain locations within **GFP** tolerate insertion of entire proteins, and conformational changes in the inserted protein can be found effects on the fluorescence. For example, insertion of the calmodulin binding domain from [illegible] Tyr-145 of a yellow mutant enhanced yellow fluorescent protein of **GFP** paralleled by increased fluorescence can be enhanced

[illegible] completed by David O'Hallaran [illegible]

Mar [illegible]

[illegible] by a metal binding. The fluorescent [illegible] in the presence of metal ions. The fluorescent [illegible] well. The [illegible] of PFP is [illegible] and the [illegible] the [illegible] is comparatively rigid and [illegible] the [illegible] [illegible].

L41 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1998:[illegible] HCAPLUS
DOCUMENT NUMBER: 128:[illegible]
TITLE: Fluorescent [illegible] green fluorescent protein and [illegible] the detection of analytes
INVENTOR(S): **Tsien, Roger Y.**; Miyawaki, Atsushi
PATENT ASSIGNEE(S): The Regents of the University of California, USA
SOURCE: PCT Int. Appl., [illegible] pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO [illegible]	A1	[illegible]	WO [illegible]	[illegible]
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG
US [illegible]	A	[illegible]	US 1997-[illegible]	19970314
US 6146826	B1	20001114	US 1997-818252	19970314
AU [illegible]	A1	[illegible]	AU 1998-67023	19980313
EP [illegible]	A1	[illegible]	EP 1998-907175	19980313
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
US [illegible]	A1	[illegible]	US [illegible]	[illegible]

PRIORITY APPLN. INFO.:
US 1997-818252 A 19970314
US 1997-818253 A 19970314
US 1997-919141 A 19970827
WO 1998-US4978 W 19980313

AB Fluorescent indicators including a binding protein moiety, a donor fluorescent protein moiety, and an acceptor fluorescent protein moiety are described. The binding protein moiety has an analyte-binding region which binds an analyte and causes the indicator to change conformation upon [illegible] the analyte. The donor moiety and the acceptor moiety change position relative to each other when the analyte binds to the analyte-binding region. The donor moiety and the acceptor moiety exhibit fluorescence resonance energy transfer when the donor moiety is excited and the distance between the donor moiety and the acceptor moiety is small. The indicators can be used to measure analyte concns. in samples, such as [illegible] in cells. For example, a fluorescent indicator for Ca was prepd. by sandwiching a Xenopus calmodulin-like [illegible] between a blue fluorescent [illegible] and a **green** fluorescent [illegible] mutant. The **green** fluorescent protein **GFP**, wherein Ala is [illegible] the chromophore, also exhibits pH sensitivity at physiol. [illegible]. Mammalian cells [illegible] expressing a polyhistidine tag were

[illegible] expression in [illegible] cells, purified by nickel-chelate and size-exclusion chromatography, and their fluorescence characterized. [illegible] emission [illegible] by [illegible] and [illegible] the [illegible] calmodulin, the ratio of [illegible] nm to 440 nm emission [illegible] increased by [illegible] upon binding [illegible]. Improved expression in mammalian cells is achieved by [illegible] mammalian [illegible] into the cDNA and mutating [illegible], and [illegible].

REFERENCE COUNT: [illegible] THERE ARE [illegible] CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L[illegible] ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT [illegible] ACS

ACCESSION NUMBER: [illegible] HCAPLUS

DOCUMENT NUMBER: [illegible]

TITLE: [illegible] assay, substrates for proteinases with cleavage sites flanked by moieties that exhibit fluorescence resonant energy transfer

INVENTOR(S): **Tsien, Roger Y.**; Heim, Roger; Cubitt, Andrew

PATENT ASSIGNEE(S): Regents of the University of California, USA; Aurora Biosciences Corp.

SOURCE: PCT Int. Appl., [illegible] pp. CODEN: [illegible]

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: [illegible]

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO [illegible]	A1	[illegible]	WO 1997-US1457	19970131
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU [illegible]	A1	[illegible]	AU 1997-22504	19970131
AU [illegible]	B2	[illegible]		
EP [illegible]	A1	19981118	EP 1997-905667	19970131
EP [illegible]	B1	[illegible]		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
AT [illegible]	E	[illegible]	AT 1997-905667	19970131
ES [illegible]	T3	20021216	ES 1997-905667	19970131

PRIORITY APPLN. INFO.: US 1996-594575 A 19960131; WO 1997-US1457 W 19970131

AB [illegible] peptide assay substrates for proteinases are described. The peptides are derived from the cleavage site of the proteinase and are flanked by [illegible] and acceptor groups. The donor and acceptor moieties exhibit fluorescence resonance energy transfer (FRET) that is eliminated upon cleavage. The fluorogenic groups may be added to the peptide [illegible] or the cleavage peptide may be part of a larger protein that exhibits natural fluorescence resonance energy transfer, e.g. a deriv. of **green** [illegible] protein. A pair of peptides exhibiting FRET [illegible] assay many cleavage reactions where the group of interest [illegible]. A **fusion protein** of a **green fluorescent protein** and a blue

Ran [illegible]

[illegible] protein analog connected by a linker that includes [illegible] sites for trypsin, chymotrypsin and enterokinase was prepared by expression from a [illegible] gene. The **fusion protein** shows intact **green fluorescence**. When treated by trypsin, the **green** [illegible] disappears with complete and [illegible] first step by these [illegible]. The [illegible] **green** and [illegible] by these [illegible] Mutants in [illegible] [illegible] that [illegible] [illegible] trypsin.

L[illegible] ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT [illegible] ACS on STN
ACCESSION NUMBER: [illegible]:[illegible] HCAPLUS
TITLE: Fluorescent biosensors of intracellular signal transduction: Applications to pharmaceutical screening.
AUTHOR(S): **Tsien, R. Y.**; Heim, R.; Taitt, A. B.; Blackman, S.; Schmaltz, T. E.
CORPORATE SOURCE: Howard Hughes Med. Inst., UCSD, La Jolla, CA, 92093, USA
SOURCE: Abstracts, 213th ACS Natl Meet, San Francisco, April 13-17, 1997, BIOL-474. American Chemical Society: Washington, D. C.
CODEN: 66AAAA
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB Fluorescent proteins as intracellular signal transducers, together with microinjection, electroporation, cell lines and miniaturized parallel assays, provide many promising approaches to ultra-high-throughput screening of pharmaceutical libraries. A) **Proteins** can be **fluorescently** labeled in vivo by **fusion** to **Green Fluorescent Protein** (**GFP**). Mutagenesis, guided in part by the crystal structure, has improved **GFP** and yielded new colors, which reveal protein-protein interaction and protease activity by fluorescence resonance energy transfer (FRET). B) Gene expression can be visualized in single living cells by using .beta.-lactamase as a reporter enzyme. It cleaves novel membrane-permeant substrates and changes their fluorescence from **green** to blue by disrupting FRET. Because of its enzymic amplification and nondestructive assay, .beta.-lactamase has major advantages over existing reporter genes. C) A fast and sensitive optical readout of membrane voltage results from FRET between a fluorescent cationic charge and another fluorophore attached to one side of the membrane.

=> d his
L1 [illegible] SEA TSIEN R?/AU
L2 [illegible] SEA CAMPBELL R?/AU
L3 [illegible] SEA L1 OR L2
L4 [illegible] SEA (GREEN(3A)FLUORESCENT(3A)PROTEIN#
L5 [illegible] SEA L4 AND L3
L6 [illegible] SEA (CHIMERIC(3A)FLUORESCENT(3A)PROTEIN#
L7 [illegible] SEA L6 AND L3
L8 [illegible] SEA (COVALENT(3A)FLUORESCENT(3A)PROTEIN#
L9 [illegible] SEA (FUSION OR FUSED OR CHIMERIC OR CHIMERI?)(3A)(FLUORESCENT(3A)PROTEIN#
L10 [illegible] SEA L1 AND L3
L11 [illegible] SEA L2 OR L7 OR L10) AND (GREEN(3A)GFP
L12 [illegible] SEA (MULTIMERIC(3A)FLUORESCENT(3A)PROTEIN#
L13 [illegible] SEA (N-TERMINUS OR N-TERMINAL OR NH2)(3A)(FLUORESCENT(3A)PROTEIN#

Search completed by Carol J Christner [illegible]

L[illegible], [illegible]

[illegible], Mikhaylenko-Maklaya [illegible], Moscow [illegible], Russia.
JOURNAL: [illegible]
[illegible]
PUB. COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: [illegible]
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
[illegible]
[illegible]

AB [illegible] applications [illegible] **green** fluorescent protein [illegible] **GFP**' -like fluorescent proteins (FPs) [illegible] the class [illegible] tend [illegible] to form **oligomeric** [illegible]. Here we suggest a strategy to [illegible] this problem by the use of two genetically linked identical red FPs or **non-oligomerizing** fusion tags. We have applied this approach to the **dimeric** [illegible] **fluorescent protein** [illegible] to study the subcellular localization of the [illegible] in living mammalian cells [illegible] cytoskeletal structures and tiny [illegible]. In addition, a possibility [illegible] fluorescence resonance energy transfer (FRET) between enhanced yellow FP mutant (EYFP) and mutant RFP1 was demonstrated in a protease assay.

L[illegible] ANSWER [illegible] OF [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Intracellular localization of Herpes simplex virus type 1 thymidine kinase fused to different fluorescent protein variants in living cells and its effect on the resident cell.
AUTHOR: Selling Ariane; Simm Andreas; Bernhard Nikolai
CORPORATE SOURCE: Molecular Neurooncology Laboratory, Department of Neurosurgery, Martin-Luther-University Halle-Wittenberg, Heinrich-Lamerow-Strasse 1, [illegible], Halle, Germany..
ariane.selling@medizin.uni-halle.de
SOURCE: FEBS LETTERS, (2002 Sep 11) 527 (1-3) 153-8.
Journal code: [illegible]. ISSN: [illegible].
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: 20020911
Last Updated on STN: 20021213
Entered Medline: 20020911

AB Gene therapy employing the suicide gene prodrug activating system Herpes simplex virus type 1 thymidine kinase (HSV-TK)/ganciclovir (GCV) is [illegible] in malignant cells. Labelling of the HSV-TK enzyme with fluorescent proteins makes possible the non-invasive imaging of transduction efficiency, enzyme localization and activity in cell culture and in animal models of human cancers. Here we report the expression of HSV-TK fused with different fluorescent proteins (EYFP, DsRed1, Isabel, [illegible]) and show that intracellular localization of the fusion proteins is not altered by the fluorescent tag despite the presence of several nuclear localization signals within the enzyme itself. Co-expression of the resistant HSV-TK fusion proteins with TK-EYFP or enhanced HSV-TK all these proteins to enter the nucleus by inhibiting formation of **fluorescent protein oligomers**. As enzyme localization may influence HSV-TK activity, this observation is [illegible]

[illegible] computer by Paula Schreiber [illegible]

Entered Medline: [illegible]

AB **Oligomerization** [illegible] is [illegible] of β-pleated-sheet peptides [illegible] has been reported as an important factor in amyloid formation. This concept has recently gained widespread interest due to the application [illegible] and biochemical techniques such as fluorescence resonance energy transfer (FRET), which have shown dramatically that several types of [illegible] can form stable **oligomers** in living cells. Current challenges are to determine which [illegible] are [illegible] with other [illegible], to define the molecular principles that govern these specific interactions, and to correlate which specific [illegible] **oligomerization**. Although these questions are [illegible] by using [illegible] expressed exclusively in their native cell types, analysis of [illegible] **oligomerization** in [illegible] model expression systems will be useful to survey which [illegible] can interact, to conduct structure/function studies, and to identify peptides or small molecules that disrupt [illegible] **oligomerization** in [illegible]. Here, we describe methods employing spectrally [illegible] resonance energy transfer (SRET) between [illegible] tagged with enhanced cyan and yellow fluorescent proteins (CFP and YFP) in living yeast cells. This approach provides a powerful means to analyze **oligomerization** of a variety of [illegible] that can be expressed in yeast, such as adrenergic, [illegible], [illegible], muscarinic acetylcholine, [illegible], [illegible], and [illegible] receptors.

L1 ANSWER [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: **Oligomerization** of [illegible] is required for the [illegible] of a functional red fluorescent chromophore.
AUTHOR: Jakobetti Andrea; Subramaniam Vinod; Jovin Thomas M; Alberti Saverio
CORPORATE SOURCE: Laboratory of Experimental Oncology, Department of Cell Biology and Oncology, Istituto di Ricerche Farmacologiche Mario Negri-Consorzio Mario Negri Sud, 66030, Chieti, Santa Maria Imbaro, Italy.
SOURCE: FEBS LETTERS, (2002 Aug 14) 525 (1-3) 13-9.
Journal code: [illegible]. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB The [illegible] fluorescent protein (DsRed) absorbs and emits light at much higher wavelengths than the structurally homologous **green** fluorescent protein, raising questions about the properties of its chromophore. We have analyzed the relationship between the appearance of red fluorescence of native, 6-histidine-tagged, or maltose-binding protein-fused DsRed. In all cases, newly synthesized DsRed molecules were initially non-red and devoid of spectrally classical chromophores. Maturation in vivo induces the expression of red **fluorescent** chromophore exclusively in **oligomeric** forms of the **protein**, whereas monomers are essentially devoid of fluorescence. NaOH-denatured samples demonstrated a generalized breakdown of the fully-red **oligomers** to monomers, which refolded after neutralization into weakly **green** fluorescent and still non-mature species. Red fluorescent chromophores were reconstituted only upon **oligomerization**. These findings demonstrate that 'true'

[illegible] oligomers, and
[illegible] the smallest [illegible] that [illegible]. A
[illegible] [illegible], and [illegible] [illegible] [illegible]
[illegible] oligomerization may play a [illegible] [illegible]
[illegible].

[illegible] MEDLINE
[illegible] MEDLINE
[illegible]
TITLE: Oligomerization of green
fluorescent protein in the secretory
pathway of endocrine cells.
AUTHOR: [illegible]
[illegible] [illegible], [illegible] and [illegible],
[illegible]
[illegible]
JOURNAL NUMBER: [illegible]
CODEN: [illegible]
[illegible]
PUB. NUMBER: [illegible]
[illegible]
LANGUAGE: English
FILE SEGMENT: [illegible]
ENTRY MONTH: [illegible]
ENTRY DATE: [illegible]
[illegible]
[illegible]

AB Green [illegible] protein GFP is used extensively as
a [illegible] to monitor cellular processes, including intracellular
[illegible] and secretion. In general, this approach depends on
GFP acting as a passive reporter protein. However, it was
recently noted that GFP oligomerizes in the secretory
pathway of endocrine cells. To characterize this oligomerization
and its potential role in GFP transport, cytosolic and secretory
forms of enhanced GFP (EGFP) were expressed in GH4C1 and AtT-20
endocrine cells. Biochemical analysis showed that cytosolic EGFP existed
as a [illegible] monomer, whereas secretory forms of EGFP formed
[illegible] oligomers. EGFP contains two cysteine
residues, Cys[illegible] and Cys[illegible], which could play a role in this
oligomerization. Site-directed mutagenesis of Cys[illegible] and Cys[illegible]
showed that both cysteine residues were involved in disulphide
interactions. Substitution of either cysteine residue resulted in a
reduction or loss of oligomers, although dimers of the secretory
forms of EGFP remained. Mutation of these residues did not adversely
affect the fluorescence of EGFP. EGFP oligomers were stored in
secretory granules and secreted by the regulated secretory pathway in
[illegible] AtT-20 cells. Similarly, the monomeric form of EGFP was
still secreted via the regulated secretory pathway, indicating that the
higher-order oligomers were not necessary for sorting in AtT-20
cells. These results suggest that the oligomerization of EGFP
must be taken into account when the protein is used as a reporter molecule in the
secretory pathway.

[illegible] MEDLINE
[illegible] MEDLINE
[illegible]
TITLE: Oligomerization of the human thyroid 5-HT
[illegible] fluorescent protein-[illegible]

[illegible] [illegible] by [illegible]

L[illegible]

TITLE: [illegible] thyroid stimulating hormone.
AUTHOR: [illegible]
CORPORATE SOURCE: [illegible]
SOURCE: [illegible]
PUB. TYPE: [illegible]
DOCUMENT TYPE: [illegible]
LANGUAGE: [illegible]
FILE SEGMENT: [illegible]
ENTRY MONTH: [illegible]
ENTRY DATE: [illegible]

AB [illegible] green fluorescent protein (GFP) and the corresponding chimeric cDNA [illegible]. Fluorescent TSH receptor [illegible] GFP-tagged TSH receptors were [illegible]. The binding of TSH, as well as TSHR autoantibodies, [illegible]. Fluorescence resonance energy transfer between receptors differentially tagged with GFP variants (CFP and YFP) provided evidence for the close proximity of [illegible] receptor molecules. This was consistent with previous studies demonstrating the presence of TSHR dimers and oligomers in thyroid tissue. Co-immunoprecipitation of GFP-tagged and Myc-tagged receptor complexes was performed using doubly transfected cells with Myc antibody. Western blotting of the immunoprecipitated complex revealed the absence of noncleaved TSH holoreceptors. This further suggested that cleavage of the holoreceptor into its two-subunit structure, comprising disulfide-linked TSHR-alpha and TSHR-beta subunits, was required for the formation of TSHR dimers and higher order complexes.

L[illegible] ANSWER [illegible] OF [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Self-assembly and binding of a sorting nexin to sorting endosomes.
AUTHOR: [illegible]
CORPORATE SOURCE: [illegible]
SOURCE: [illegible]
PUB. TYPE: [illegible]
DOCUMENT TYPE: [illegible]
LANGUAGE: [illegible]
FILE SEGMENT: [illegible]
ENTRY MONTH: [illegible]
ENTRY DATE: [illegible]

[illegible] completed by Latrice Schreiber [illegible]

Page ...,...

AB [illegible] green fluorescent protein fusions, cytolytic chromophore markers and [illegible] green fluorescent protein-DNMI. Fluorescence anisotropy transfer indicated that spectral variants of green fluorescent protein-DNMI were oligomeric in vivo. In contrast, those green fluorescent protein-DNMI oligomers dissociated into tetramers and monomers upon dilution. Green fluorescent protein-DNMI, but not green fluorescent protein-DNMI, could be photobleached to a stable dark state by illumination with blue light. Photobleaching of green fluorescent protein-DNMI was used to mark a subset of mitochondria in living cells. These results demonstrate that green fluorescent protein-DNMI will be useful for studying the dynamics of mitochondrial morphology, but that it should not be employed where a rapidly diffusible DNMI will be required. [illegible] green fluorescent protein fusions, cytolytic chromophore markers and [illegible] green fluorescent protein-DNMI. Fluorescence anisotropy transfer indicated that spectral variants of green fluorescent protein-DNMI were oligomeric in vivo. In contrast, those green fluorescent protein-DNMI oligomers dissociated into tetramers and monomers upon dilution. Green fluorescent protein-DNMI, but not green fluorescent protein-DNMI, could be photobleached to a stable dark state by illumination with blue light. Photobleaching of green fluorescent protein-DNMI was used to mark a subset of mitochondria in living cells. These results demonstrate that green fluorescent protein-DNMI will be useful for studying the dynamics of mitochondrial morphology, but that it should not be employed where a rapidly diffusible DNMI will be required.
http://www.pnas.org/cgi/content/full/97/22/11984

L... ANSWER 1 OF 24 MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Photochemistry, mutagenesis, and oligomerization of [illegible]-Ds-Red, a red fluorescent protein from coral.
AUTHOR: Baird G S; Zacharias D A; Tsien R Y
CORPORATE SOURCE: Department of Pharmacology, University of California, San Diego, La Jolla, CA 92093, USA.
CONTRACT NUMBER: [illegible] (NINDS)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, [illegible] Oct 24 97 (22) 11984-9.
Journal code: [illegible]. ISSN: [illegible].
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]
AB [illegible]-Ds-Red is a recently cloned 28-kDa red fluorescent protein responsible for the red coloration around the oral disk of a coral of the Discosoma genus. [illegible]-Ds-Red has attracted tremendous interest as a potential expression tracer and fusion partner that would be complementary to the numerous green fluorescent protein from Aequorea, but very little is known of the photochemistry of [illegible]-Ds-Red. We now show that [illegible]-Ds-Red has a much higher extinction coefficient and quantum yield than previously reported, plus substantial resistance to pH extremes and proteolysis. In addition, the excitation maximum can be further shifted to 591 nm by mutation of Lys-83 to His. However, [illegible]-Ds-Red has many drawbacks, such as strong oligomerization and slow maturation. Analytical [illegible] of pH dependence by an added tetramer in vitro, and

Journal compiled by Lavia Schreiber [illegible]

Page [illegible]

[illegible] oligomerization in live cells. Also, fusion [illegible] green [illegible] in vitro it is dimeric, and [illegible] to [illegible] to dissociate the dimer. Many [illegible] cell [illegible] applications [illegible] require suppression of the tetramerization and acceleration of the maturation.

L [illegible] ANSWER [illegible] OF [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Determination of the frequency of [illegible] recombination between two identical sequences within a provirus.
AUTHOR: Hu J; Temin J
CORPORATE SOURCE: Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY [illegible], USA.
SOURCE: JOURNAL OF VIROLOGY, [illegible] Aug [illegible] [illegible]-[illegible].
Journal code: [illegible]. ISSN: [illegible]-[illegible]X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB [illegible] RNA as their genetic material within virus particles and DNA proviruses as their genetic material within cells. The rate of recombination during reverse transcription between two identical sequences within the same RNA molecule is very high. In this study, we have developed a sensitive system to study recombination occurring within the proviral sequence. This system includes a murine Moloney leukemia virus vector which contains a neomycin resistance gene (neo) and two mutated green fluorescent protein genes (gfp) in tandem positions. The 3' end of the first gfp and the 5' end of the second gfp gene are both mutated, so that neither of these two gfp genes is functional. However, if recombination occurs between the two gfp genes it will create a functional gfp gene. Cells containing such a functional recombinant gfp appear green under fluorescence microscopy. The rate of recombination between the two gfp sequences during a single round of replication is as high as [illegible]. Green cells appear during proliferation of proviral clear-cell population and allow a small portion of these recombinations between sequences of proviral DNA to be detected. The frequency of recombination of the proviral DNA level is about [illegible] of events per cell division, which is very low compared with the frequency of recombination [illegible] caused by reverse transcriptase and/or RNA polymerase II.

L [illegible] ANSWER [illegible] OF [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Effect of N-terminal alpha-helix formation on the intracellular and intravirion [illegible] activity of HIV-1 integrase.
AUTHOR: Lee M S; Craigie R; Laughton C A
CORPORATE SOURCE: MRC Laboratory for Molecular Cell Biology and the

Last updated by David Schreiber [illegible]-429x

Page [illegible]

[illegible] Department of Biology, University College London, London, UK.
[illegible] Network, London WC1E 6BT, United Kingdom.
Source: JOURNAL OF BIOLOGICAL CHEMISTRY, [illegible] Vol. [illegible]
[illegible].
Journal code: [illegible]. ISSN: [illegible].
Publication Country: United States
Document Type: Journal; Article; (JOURNAL ARTICLE)
Language: English
File Segment: Priority Journals
Entry Month: [illegible]
Entry Date: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB The [illegible] peroxisome-to-mitochondrion mistargeting of human liver alanine:glyoxylate aminotransferase (AGT) in the hereditary disease primary hyperoxaluria type 1 is caused by the combined presence of a common Pro11 --> Leu polymorphism and a disease-specific Gly170 --> Arg mutation. The Pro11 --> Leu replacement generates a functionally weak N-terminal mitochondrial targeting sequence (MTS), the efficiency of which is dramatically reduced by the additional presence of the Gly170 --> Arg replacement. AGT dimerization is an inhibitor of the peroxisome-to-mitochondrion [illegible] which each is present separately. In this paper we have attempted to identify the structural determinants of AGT dimerization and its role in mitochondrial mistargeting. Unlike most MTSs, the polymorphic MTS of AGT has little tendency to adopt an alpha-helical conformation in vitro. Nevertheless, it can direct efficient targeting of a monomeric **green fluorescent** GFP) fusion **protein**, but not a **dimeric** AGT, to mitochondria in transfected COS-1 cells. Increasing the propensity of this MTS to fold into an alpha-helix, by making a Pro11 --> Leu + Pro10 --> Leu replacement, enabled it to target both GFP and AGT efficiently to mitochondria. The double Pro11 --> Leu + Pro10 --> Leu replacement retarded AGT dimerization in vitro as did the disease-causing double Pro11 --> Leu + Gly170 --> Arg replacement. These data suggest that N-terminal alpha-helix formation is more important for maintaining AGT in a conformation (i.e. monomeric) compatible with mitochondrial import than it is for the precision of mitochondrial targeting information. The parallel effects of the Pro11 --> Leu and Gly170 --> Arg replacements in the dimerization and intracellular targeting of polymorphic AGT containing the Pro11 --> Leu replacement raise the possibility that they might achieve their effects by the same mechanism.

L[illegible] ANSWER [illegible] OF [illegible] MEDLINE
ACCESSION NUMBER: 97446032 MEDLINE
DOCUMENT NUMBER: 97446032 PubMed ID: 9244659
TITLE: **Oligomerization** of expanded-polyglutamine domain **fluorescent** fusion **proteins** in mammalian cells.
AUTHOR: Kimura Y; Burke J R; Miller J E; Hester J; Tenbrink J; Hynes A J; Strittmatter W J
CORPORATE AUTHOR: Department of Medicine, Neurology, Duke University Medical Center, Durham, North Carolina 27710, USA.
CONTRACT NUMBER: [illegible]-[illegible]-[illegible] (NINDS)
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, [illegible] [illegible] [illegible] [illegible] [illegible].
Journal code: [illegible]. ISSN: [illegible].
Publ. Country: United States
Document Type: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

[illegible] [illegible] by [illegible] [illegible]

[illegible]

FILE SEGMENT: [illegible]
ENTRY NUM: [illegible]
ENTRY DATE: [illegible]
[illegible]
[illegible]
[illegible]

AB [illegible] **oligomerization** [illegible]
oligomerization [illegible]
green [illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]
oligomerization [illegible]
[illegible]

[illegible]
ACCESSION NUMBER: [illegible]
DOCUMENT NUMBER: [illegible]
TITLE: Systemically delivered antisense oligomers upregulate gene expression in mouse tissues
AUTHOR(S): Nazarri, Peter; Gemignani, Federica; Kang, Shin-Hong; Maier, Martin A.; Manoharan, Muthiah; Persmark, Magnus; Bortner, Donna; Kole, Ryszard
CORPORATE SOURCE: Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC, 27599, USA
SOURCE: Nature Biotechnology (2001), 19(4), 333-336
CODEN: NABIF9; ISSN: 1087-0156
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Systemically injected 2'-O-methoxyethyl (2'-O-MOE)-phosphorothioate and PNA-4K oligomers (peptide **nucleic** acid with four lysines linked at the C terminus) exhibited sequence-specific antisense activity in a no. of mouse organs. Morpholino oligomers were less effective, whereas PNA oligomers with only the lysine (PNA-1K) were completely inactive. The latter result indicates that the four-lysine tail is essential for the antisense activity of PNA oligomers in vivo. These results were obtained in a transgenic mouse model designed as a pos. readout test for activity, delivery, and distribution of antisense oligomers. In this model, the expressed gene EGFP-654 encodes enhanced **green** fluorescence protein (EGFP) is interrupted by an aberrantly spliced mutated intron of the human beta-globin gene. Aberrant splicing of this intron prevents expression of EGFP-654 in all tissues, whereas in tissues and organs that take up splice-correcting antisense oligos, correct splicing was restored and EGFP-654 expression upregulated. The sequence-specific ability of PNA-4K and the 2'-O-MOE oligomers to upregulate EGFP-654 provides direct evidence that systemically delivered, short, modified

[illegible]

[illegible] ANSWER 1 OF 1 HCAPLUS COPYRIGHT [illegible] ACS

ACCESSION NUMBER: [illegible] HCAPLUS

DOCUMENT NUMBER: 136:[illegible]

TITLE: **dimeric fluorescent protein** [illegible]

INVENTOR(S): [illegible]

PATENT ASSIGNEE(S): [illegible]

SOURCE: PCT Int. Appl., [illegible] pp.

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

PRIORITY APPLN. INFO.: [illegible]

AB The [illegible] relates to proteins or polypeptides that comprise intramol. **dimers** of **fluorescent protein** monomers. More specifically, the invention relates to recombinant polypeptides comprising a mol. of a fluorescent polypeptide, a linker peptide, and a second monomer of that fluorescent polypeptide, where the monomers form an intramol. dimer. The invention also relates to **nucleic** acids encoding intramol. **Dimer Fluorescent Proteins** (DFPs) and to cells comprising such a **nucleic** acids. The invention further relates to methods of making DFPs and methods of using them. DFPs are useful in any application suited for fluorescent proteins and are particularly useful in applications in which more than one **fluorescent protein** sharing a polypeptide **dimerization** interface is present in the same mixt. It is expressed in the same cell, because DFPs do not form hetero-dimers.

[illegible] ANSWER 1 OF 1 HCAPLUS COPYRIGHT [illegible] ACS

ACCESSION NUMBER: [illegible] HCAPLUS

DOCUMENT NUMBER: 136:[illegible]

TITLE: [illegible]

[illegible]

Mar [illegible], [illegible]

INVENTOR(S): [illegible]
PATENT ASSIGNEE(S): [illegible]
SOURCE: [illegible]
CODEN: [illegible]
DOCUMENT TYPE: [illegible]
LANGUAGE: [illegible]
FAMILY ACC. NUM. COUNT: [illegible]
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	PI: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, R			
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	M: [illegible]			
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR			
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

PRIORITY APPLN. INFO.: [illegible]

AB The present invention relates to **nucleic** acids encoding HCV **[illegible]** interacting main polypeptides: minimal fragments of hepatitis C virus proteins responsible for specific interactions with proteins of the virus. These peptides and their interactions may be targets for prevention and treatment of infection of hepatitis C virus. The invention also includes vectors comprising a **nucleic** acid encoding a HCV polypeptide as well as host cells transformed with such vectors. The invention is also directed to yeast or bacterial two-hybrid methods which make use of the **nucleic** acids encoding a HCV polypeptide selected from a pathogenic strain of the hepatitis C virus as well as to methods for selecting mols. which inhibit the binding between a HCV polypeptide and a polypeptide which specifically binds thereto. Protein-protein interactions of HCV polypeptides may be detected by **covalent** or non-**covalent** attachment of **fluorescent proteins**, labeled antibodies or enzymes with catalytic activity to the HCV polypeptide 'marker protein' and subsequently contacting the marker protein with a plurality of other proteins like HCV polypeptides. The HCV polypeptides may also be covalently linked to a spacer, which may also be covalently bound to a substrate to immobilize the HCV polypeptides. For example, the HCV polypeptide may be attached to biotin when the substrate is streptavidin. Changes in optical properties of the substrate, following binding by HCV polypeptides are detected. HCV polypeptides can be used in vaccines for prevention and treatment of hepatitis C virus in animals or humans.

L[illegible] ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: [illegible] HCAPLUS
DOCUMENT NUMBER: 135:[illegible]
TITLE: Single molecule imaging of **green** fluorescent proteins in living cells: E-cadherin forms **oligomers** on the free cell surface
AUTHOR(S): Iino, Ryota; Koyama, Ikuko; Kusumi, Akihiro
CORPORATE SOURCE: Kusumi Membrane Organizer Project, Exploratory Research for Advanced Technology Organization, Japan Science and Technology Corporation, Nagoya, [illegible], Japan
SOURCE: Biophysical Journal, [illegible], [illegible]
CODEN: BIOJAU; ISSN: [illegible]

[illegible] completed by Kevin Schreiber [illegible]

[illegible], [illegible]

PUBLISHER: [illegible]
DOCUMENT TYPE: Journal
LANGUAGE: English
AB [illegible] **green** [illegible] **GFP** [illegible]. [illegible] **GFP** [illegible] **GFP** [illegible] **GFP** [illegible] **oligomers** [illegible] **oligomerization** [illegible] **oligomerization**. [illegible] **oligomerization**, an **oligomerization**-induced [illegible] **oligomers** [illegible] **oligomers** [illegible]
REFERENCE COUNT: [illegible] THERE ARE [illegible] CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RB FORMAT

[illegible] ANSWER [illegible] OF [illegible] AVAILABLE COPYRIGHT [illegible] ACS
ACCESSION NUMBER: [illegible]
DOCUMENT NUMBER: [illegible]
TITLE: Collective effects in individual **oligomers** of the red **fluorescent** coral **protein** DsRed
AUTHOR(S): Cotlet, M.; Hofkens, J.; Kohn, F.; Michiels, J.; Dirix, G.; Van Guyse, M.; Vanderleyden, J.; De Schryver, F. C.
CORPORATE AUTHOR: Department of Chemistry, Katholieke Universiteit Leuven, Heverlee, [illegible]
SOURCE: Chemical Physics Letters [illegible], [illegible], [illegible]
CODEN: CHPLBC; ISSN: [illegible]
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We report on single-mol. expts. that were performed on two biol. systems, the recently cloned red fluorescent protein of a coral of the Discosoma genus (DsRed) and the enhanced **green** fluorescent protein, both of which were immobilized in polyvinyl alc. Fluorescence intensity trajectories were recorded and compared to those of a red. sym., 1,6-bis(2,4,6-trimethylphenyl)perylene-3,4:9,10-bis(dicarboximide). Evidence was found for the presence of DsRed as an **oligomer** even at sub-nanomolar concns. Both systems show triplet blinking in the millisecond time range. Collective effects could be used for the DsRed **oligomers**.
REFERENCE COUNT: [illegible] THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

[illegible] ANSWER [illegible] OF [illegible] AVAILABLE COPYRIGHT [illegible] ACS
ACCESSION NUMBER: [illegible]
DOCUMENT NUMBER: [illegible]
TITLE: **Oligomerization** of the human [illegible]

Search completed by David Schreiber [illegible]

Page 2-a, [illegible]

transporter and of the rat GABA transporter 1 visualized by fluorescence resonance energy transfer microscopy in living cells

AUTHOR(S): [illegible]; Kantcheva, Rositsa B.; Whiteside, Brian; Kantcheva, Zlatka; Bossuyt, Michael; Mikhaylova, Maria; Bito, Haruhiko; Hannah, Marcella H.

CORPORATE SOURCE: Department of Vascular Biology and Thrombosis Research, University of Vienna Medical School, Vienna, A-1090, Austria

SOURCE: Journal of Biological Chemistry (2006), 281(41), 30736-30743 CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Recent structural studies indicate that the serotonin transporter can form **oligomers**. We investigated whether the human serotonin transporter (hSERT) can be visualized as an **oligomer** in the plasma membrane of living cells. For this purpose, we generated fusion proteins of hSERT and spectral variants of the **green** fluorescent protein, which we call yellow fluorescent proteins, YFP and CFP, resp. When expressed in human embryonic kidney 293 cells, the resulting fusion proteins YFP-hSERT and CFP-hSERT were efficiently inserted into the plasma membrane and were functionally indistinguishable from wild-type hSERT. **Oligomers** were visualized by fluorescence resonance energy transfer microscopy in living cells using two complementary methods, i.e. photobleaching and acceptor photosensitization. Interestingly, **oligomerization** was not confined to hSERT; fluorescence resonance energy transfer was also detected between YFP- and CFP-labeled rat gamma-aminobutyric acid transporter. The bulk of serotonin transporters was found as high mol. wt. complexes upon gel filtration in detergent solns. In contrast, the monomers of CFP-hSERT and YFP-hSERT were essentially undetectable. This indicates that the homo-**oligomeric** form is the favored state of hSERT in living cells, which is not significantly affected by coincubation with transporter substrates or blockers. Based on our observations, we conclude that constitutive **oligomer** formation might be a general property of Na+/Cl--dependent neurotransmitter transporters.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2: ANSWER 2 OF 24 HCAPLUS COPYRIGHT 2006 ACS

ACCESSION NUMBER: 1998:679443 HCAPLUS

DOCUMENT NUMBER: 129:38126

TITLE: FRET: proximity imaging of **green** fluorescent protein-tagged polypeptides

AUTHOR(S): De Angelis, Lina A.; Miyawaki, Atsushi; Tomaiuolo, Paola V.; Rothman, James E.

CORPORATE SOURCE: Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021, USA

SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1998), 95(21), 12483-12488 CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We report a novel method for visualizing the interaction of

Search completed by David Schreiber on 10-Aug-06

[illegible] green fluorescent protein (GFP) [illegible]. When two GFP molecules are brought into proximity, changes in the relative intensities of green fluorescence emitted upon excitation at 400 vs. 470 nm result. These spectral changes provide a sensitive ratiometric index of the extent of GFP-GFP [illegible] that can be exploited to quantitate image homo-oligomerization or clustering processes of GFP-tagged proteins in vivo. The method, which we term proximity imaging (PRIM), complements fluorescence resonance energy transfer between [illegible] fluorescent protein [illegible] GFP [illegible], a powerful method for imaging proximity relationships between different proteins. However, unlike [illegible] energy transfer, which is a [illegible] interaction, PRIM depends on direct contact between two GFP modules, which can lead to structural perturbations and concomitant spectral changes within a module. Moreover, the precise spatial arrangement of the GFP moieties within a given dimer [illegible] the magnitude and direction of the spectral change. We have used PRIM to detect FK1012-induced dimerization of GFP fused to FK506-binding protein and clustering of glycosylphosphatidylinositol-anchored GFP on cell surfaces.

REFERENCE COUNT: [illegible] THERE ARE [illegible] CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L[illegible] ANSWER [illegible] OF [illegible] BIOSIS COPYRIGHT [illegible] BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: [illegible] BIOSIS
DOCUMENT NUMBER: [illegible]
TITLE: AAV-Mediated Delivery of Rhodopsin to the Rhodopsin Knockout Mouse Retina.
AUTHOR(S): Dejneka, N. S. (1); Liang, F.-Q. (1); Maguire, A. M. (1); Bennett, J. (1)
CORPORATE SOURCE: (1) Dept. of Ophthalmology, Scheie Eye Institute, University of Pennsylvania, Philadelphia, PA, USA USA
SOURCE: ARVO Annual Meeting Abstract Search and Program Planner, (2002) Vol. 2002, pp. Abstract No. 460[illegible]. cd-rom. Meeting Info.: Annual Meeting of the Association For Research in Vision and Ophthalmology Fort Lauderdale, Florida, USA May [illegible], 2002
DOCUMENT TYPE: Conference
LANGUAGE: English

AB Purpose: Rhodopsin knockout mice (rho-/-) lack outer segments, and photoreceptor degeneration is nearly complete by post-natal day [illegible] (Lem et al. 1999 PNAS, 96: 736-741). This study aimed to deliver rhodopsin to these animals via an adeno-associated virus 2 (AAV2), and assess potential rescue effects. Methods: An AAV2, expressing the murine rhodopsin cDNA in **tandem** with the marker enhanced **green fluorescent protein** was prepared. Rho-/- mice (post-natal days 3-9) subsequently received the virus subretinally (single eye) and were maintained for 12 weeks. Contralateral eyes served as untreated controls. Five, 8 and 12 weeks post-injection, eyes were enucleated, fixed, cryoprotected and assessed histologically. Samples were analyzed for rhodopsin (primary antibody, rho4D2) and EGFP expression, as well as for evidence of photoreceptor rescue, using fluorescent microscopy. Results: Five weeks post-injection, EGFP expression localized to photoreceptor cell bodies and inner segments. Immunohistochemistry revealed strong rhodopsin labeling localized to the tips of the inner segments. A similar expression pattern was also evident at 8 weeks post-treatment. At this time there also appeared to be focal rescue of photoreceptors surrounding the site of injection; however, there was no evidence of outer segment formation, and the rescue effect was not

[illegible]

[illegible] by [illegible] of the fluorescent [illegible] that [illegible] initially, and [illegible] path and EGFP expression was [illegible] without [illegible] [illegible]: DAPI-mediated multiply [illegible] analysis [illegible] [illegible] approach [illegible] [illegible] the [illegible] phenotype. Double [illegible] [illegible] [illegible] multiply [illegible]. [illegible] that it [illegible] expression [illegible] [illegible] that [illegible] [illegible] [illegible] [illegible] [illegible] as well as the [illegible] [illegible] [illegible] [illegible] transfection [illegible]. [illegible] the [illegible] [illegible] [illegible] [illegible] [illegible] and [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible].

ACCESSION NUMBER: [illegible] BIOSIS
DOCUMENT NUMBER: PREV[illegible]
TITLE: [illegible]-FRET microscopy in living cells to measure [illegible] homo-dimer translocation of **GFP**-tagged proteins.
AUTHOR(S): [illegible], I.; Tramier, M.; Durieux, C.; Coppey, J.; Pansu, R. B.; Nicolas, J.-C.; Kemnitz, K.; Coppey-Moisan, M.
CORPORATE AUTHOR: [illegible] Institut Jacques Monod, UMR [illegible], [illegible] Paris [illegible] [illegible] Paris, France
SOURCE: Biophysical [illegible], [illegible], [illegible] [illegible], [illegible], [illegible]. [illegible]. print.
ISSN: [illegible].
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Fluorescence anisotropy decay microscopy was used to determine, in individual living cells, the spatial homo-dimer distribution of proteins, advantageously fitted by herpes simplex virus thymidine kinase (TK) fused to **green** fluorescent protein (**GFP**). Accordingly, the fluorescence anisotropy dynamics of two fusion proteins (TK-**GFP** and TK(3)-**GFP**) was recorded in the confocal mode by ultra-sensitive time-correlated single-photon counting. This provided a measurement of the rotational time of these proteins, which, by comparing with **GFP**, allowed the determination of their **oligomeric** state in both the cytoplasm and the nucleus. It also revealed energy homo-transfer within aggregates that TK-**GFP** progressively formed. Using a symmetric dimer model, structural parameters were estimated; the mutual orientation of the transition dipoles of the two **GFP** chromophores, calculated from the residual anisotropy, was 44.6+-1.0degree, and the upper intermolecular limit between the two fluorescent tags, calculated from the energy transfer rate, was [illegible] ANG. Acquisition of the fluorescence steady-state intensity, lifetime, and anisotropy decay in the same cells, at different times after transfection, indicated that TK3660FP was initially in a monomeric state and then formed dimers that grew into aggregates. Fluorescence time-resolved fluorescence anisotropy microscopy opens a promising avenue for obtaining structural information on proteins in [illegible] [illegible], when [illegible] expressed [illegible] [illegible].

ACCESSION NUMBER: [illegible]:[illegible] BIOSIS
DOCUMENT NUMBER: PREV[illegible]
TITLE: A novel nuclear receptor heterodimerization pathway mediated by orphan receptors TR2 and TR4.
AUTHOR(S): Lee, Chih-Hao; Chinpaisal, Chatchai; Wei, Li-Na [illegible]
CORPORATE AUTHOR: [illegible] Dep. Pharmacol., Univ. Minnesota, [illegible] Millard Hall, 420 Delaware St. S.E., MN [illegible] USA
SOURCE: Journal of Biological Chemistry, [illegible], [illegible] [illegible], No. [illegible], pp. [illegible].

[illegible] by [illegible] [illegible]

[illegible]

ISSN: [illegible]
DOCUMENT TYPE: Article
LANGUAGE: English
AB [illegible] DNA [illegible] green fluorescent protein. [illegible] dimerization [illegible]

L[illegible] ANSWER [illegible] EMBASE COPYRIGHT [illegible] ELSEVIER B.V.
ACCESSION NUMBER: [illegible] EMBASE
TITLE: [illegible] GFP-FRET [illegible]
AUTHOR: [illegible]
CORPORATE SOURCE: [illegible]
SOURCE: [illegible]
ISSN: [illegible] CODEN: JBISF3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: [illegible] Cancer
[illegible] Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Apart [illegible] caspases also appear to be central players in several pathological states, there is a practical need within the biopharmaceutical research community for facile, noninvasive cellular assays [illegible] Tandem [illegible] green fluorescent protein GFP [illegible]

[illegible] completed by David Schreiber [illegible]

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[illegible]

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114 [illegible] ALIAS AND FULLRESIDENT [illegible]

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